DOI:
10.1089/rej.2016.1872

Document Version
Peer reviewed version

Rejuvenation Research
© Mary Ann Liebert, Inc.
DOI: 10.1089/rej.2016.1872

SERUM THYROID-STIMULATING HORMONE LEVELS AND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY

Running head: TSH and frailty

Nicola Veronese[1], MD, Sara Fernando Watutantrige [2], MD, Stefania Maggi[3], MD, Marianna Noale[3], MD, Brendon Stubbs[4,5,6], PhD, Raffaele Antonelli Incalzi[7], MD, Sabina Zambon[3,8], MD, Maria Chiara Corti[9], MD, Egle Perissinotto[10], ScD, Gaetano Crepaldi[3], MD, Enzo Manzato[1,3], MD, Giuseppe Sergi[1], MD

[1] Department of Medicine (DIMED), Geriatrics Division; University of Padova, Italy.

[2] Department of Medicine (DIMED), Endocrinology Unit, University of Padova, Italy.

[3] National Research Council, Neuroscience Institute, Padova, Italy.

[4] Health Service and Population Research Department, Institute of Psychiatry, King's College London, De Crespigny Park, London Box SE5 8AF, United Kingdom.

[5] Physiotherapy Department, South London and Maudsley NHS Foundation Trust, Denmark Hill, London SE5 8AZ, United Kingdom.

[6]Faculty of Health, Social Care and Education, Anglia Ruskin University, Bishop Hall Lane, Chelmsford CM1 1SQ, UK.

[7] Geriatrics-Unit of Respiratory Pathophysiology, Campus Bio Medico University and Teaching Hospital, Rome, Italy.

[8]Department of Medicine (DIMED), Clinica Medica I, University of Padova, Italy.

[9]Division of Health Care Planning and Evaluation of the Regione Veneto, Venice, Italy.

[10] Department of Cardiac, Thoracic and Vascular Sciences - Biostatistics, Epidemiology and Public Health Unit, University of Padova, Padova, Italy.

Address for correspondence and reprints:

Nicola Veronese

Department of Medicine - DIMED, Geriatrics Division, University of Padova, Padova, Italy

Via Giustiniani, 2 - 35128 Padova, Italy

Phone: +390498218492; Fax: +390498211218

Email: ilmannato@gmail.com

Key words: thyroid; frailty; elderly; community-dwelling adults.

Word count: 3097

ABSTRACT

High or low thyroid-stimulating hormone (TSH) levels seem to be associated with several negative outcomes in the elderly, but the literature about TSH and frailty is still limited. In this paper, we investigated whether TSH is associated with prevalent and incident frailty in a cohort of older community-dwelling subjects. Among 3,099 initially screened in the Progetto Veneto Anziani Study, 2,571 men and women aged >65 years (for cross-sectional analyses) and 1,732 (longitudinal, mean follow-up period of 4.4 years) were divided into sex specific quintiles according to baseline serum TSH concentrations within normal range (0.3 and 4.2 mUI/L). Frailty was defined as the presence of 3 among 5 Fried's criteria. At baseline, taking those in the third quintile of serum TSH as reference (Q3) and adjusting for potential confounders, participants in the highest (Q5) quintile, had an increased odds ratio (OR) of having frailty (OR=1.55; 95%CI: 1.03-2.33 for men; =1.97; 95%CI: 1.59-2.45 for women). Men in Q1 had a significant higher odds of having muscle weakness and exhaustion, whilst those in Q5 of muscle weakness and slow gait speed. Women in Q1 had a significant higher odds of having all the Fried's criteria (except for exhaustion), whilst those in Q5 reported a significant higher presence of muscle weakness and slow gait speed. At follow-up, men in Q5 had an increased risk of frailty (OR=1.37; 95%CI: 1.02-1.91) similarly to women in Q1 (OR=1.47; 95%CI: 1.21-1.78). In conclusion, men with higher and women lower serum TSH levels are at increased risk of frailty.

INTRODUCTION

Frailty is typically defined as a state of increased vulnerability to stressors that results from decreased physiologic reserve in multiple organ systems causing limited capacity to maintain homeostasis.[1] It is a highly prevalent condition in older people with an estimated prevalence of 10% in community-dwelling people.[2] Frailty is associated with several deleterious outcomes in the elderly, including higher rate of hospitalization, falls, disability and mortality [3].

There is a growing evidence base to suggest that frailty is characterized by a "metabolic signature".[4] Frail subjects are characterized by a higher chronic inflammation levels[5] and concomitant down-regulation of multiple endocrine factors[4], suggesting that these markers could be useful for the early identification of frailty.

Unfortunately, very few representative studies have assessed the potential relationship between thyroid function (assessed with thyroid-stimulating hormone [TSH]) and frailty. Two cross-sectional studies[6,7] reported a non-significant association between TSH levels and frailty, although one found an association between free thyroxine and frailty.[7] One longitudinal study [8] did not find no association of subclinical hyperthyroidism and hypothyroidism with incident frailty in older men. This longitudinal study, however, included very few participants for both hyperthyroidism (n=22) and hypothyroidism (n=85). These findings, however, are somewhat surprising since both subclinical hyperthyroidism and hypothyroidism are associated with a high rate of medical conditions significantly associated with frailty as previously mentioned.

Previous literature has suggested that in the elderly thyroid function at the extremes of the normal range could be associated with the onset of several medical conditions.[9,10] However, the knowledge of the relationship between TSH and frailty is still limited and worthy of improvement through representative research. We investigated whether TSH is associated with frailty in a cohort of older men and women. We hypothesized that assessing the association between TSH and frailty in both cross sectional and longitudinal studies of the same population and correcting for potential confounders might provide more definitive evidence about the TSH-frailty relationship.

METHODS

Data source and subjects

The data for this analysis derived from the *Progetto Veneto Anziani* (Pro.V.A.), an observational cohort study on the Italian population aged ≥65 years. The study population included 3099 age- and sex-stratified Caucasian participants (1854 women and 1245 men) randomly selected between 1995 and 1997 using a multistage stratification method. Sampling procedures and data collection methods have been described elsewhere.[11] The current study utilizes information obtained at baseline and at follow-up after 4 years.

The local ethical committees of Padova University and the Local Health Units (ULSS) n. 15 and n. 18 of the Veneto Region approved the study protocol, and participants gave their written informed consent.

Clinical data

Participants were examined at city hospitals by trained physicians and nurses. Information was collected on their smoking, alcohol drinking, education and monthly income during a face-to-face interview. Smoking status was classified as "current" vs. "never/former" (smoking for at least a year in the past). Alcohol drinking was categorized as "yes" vs. "no" in the previous month. Education was categorized as < vs. >5 years, being 5 years the level of primary education in Italy. Monthly income as > vs. < 500€. Body weight and height were measured by trained physicians and body mass index (kg/m^2) was calculated.

Disability was evaluated with the number of activities of daily living (ADL) preserved using the index proposed by Katz et al.[12], while cognitive status and depressive mood with 30-items mini-mental state examination (MMSE)[13] and geriatric depression scale (GDS)[14], respectively.

Details of all medical conditions were ascertained by board-certified physicians involved in the study, who examined all of the clinical information collected for each participant, including disease history, symptoms self-reported using standardized questionnaires, medical and hospital records,

blood tests, and physical examination.[11] For the aim of this study, validated general health measures of comorbidities were assessed by calculating modified Charlson comorbidity score, with higher scores indicating an increased severity of conditions.[15] Renal function was assessed from the estimated glomerular filtration rate (eGFR) calculated with the Modified Diet in Renal Disease formula.

Definition of exposure and outcome

Blood samples were obtained after an overnight fast for biochemical tests, which were performed at the city hospital's central laboratory using standard, quality-controlled procedures. TSH was measured in serum with a third-generation immunoassay using a range for normality values between 0.3 and 4.2 mUI/L.[16] The coefficients of variation for this test (intra and inter-assay) were less than 5%. Since a significant difference between men and women existed ($p<0.0001$, t-test independent samples), the population was divided in quintiles using for men 0.7, 1.0, 1.3, and 2.0mUI/L and for women 0.8, 1.1, 1.5, and 2.5 mUI/L as cut-offs.

Frailty was the primary outcome of interest. Fried defined frailty using 5 items (unintentional weight loss, low physical activity level, weakness, exhaustion, and slow gait speed).[17] For this research, we used a slightly modified version[18]:

-Unintentional weight loss: self-reported unintentional weight loss >5 kg over the past year without known reasons.

-Self-reported low physical activity level: weekly physical activity below 383 kcal in males and 270 kcal in females,[17] calculated from the sum of all the leisure-time activities performed during a typical week in the previous month.

-Weakness: best handgrip strength value of 3 attempts of the dominant hand below the sex and BMI specific cutoffs suggested by Fried et al.[17]

-Exhaustion: a geriatric depression scale (GDS) scale≥10/30 and a negative answer to the question: "Do you feel full of energy?" [19]

SERUM THYROID-STIMULATING HORMONE LEVELSAND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

-Slow gait speed: the best timed walk over 4 m at usual pace below the sex and BMI specific cutoffs.[17]

Subjects unable to perform the handgrip strength or walking tests were considered as having weakness or slow gait speed, respectively. Participants were classified as a) frail if they met 3 or more of the 5 modified Fried criteria, b) pre-frail if they met 1 or 2 criteria and c) non-frail (robust) if they met none of the criteria.

Statistical analyses

To generalize the Pro.V.A. sample to the general population of the two geographical areas, a set of weights was defined according to the sex and age distribution of the reference population (Italy, Census 1991) and to the sample fraction.

Since a significant interaction between TSH and gender existed taking frailty (prevalent or incident) as outcome, the data are reported according to gender. For continuous variables, normal distributions were tested using the Shapiro-Wilk test. Age-adjusted p values were calculated by TSH quintiles using a generalized linear model with the Bonferroni's correction and taking the third quintile (Q3) (containing the mean of the baseline TSH value) as the reference group. Levene's test was used to test the homoscedasticity of variances and, if its assumption was violated, Welch's ANOVA was used. Differences in categorical variables were examined using the logistic regression analysis, including age as covariates.

For baseline data, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) to assess the strength of the association between TSH and frailty, taking those in Q3 as reference. In secondary analyses, we also evaluated the association between TSH and each of the single items in Fried's criteria, taking those with any criterion as reference.

For follow-up analyses, we removed the subjects already frail at baseline and those without follow-up data, investigating the incidence of frailty by baseline TSH concentrations. The proportional hazards assumption was checked by plotting the Schoenfeld residuals versus time[20], but since the p

SERUM THYROID-STIMULATING HORMONE LEVELSAND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

value was less than 0.05 for the interaction of TSH by frailty at follow-up, a logistic regression was used instead of Cox's regression analysis.

Known factors associated with frailty were considered for inclusion in the analysis including in the fully-adjusted model all the variables reaching a $p<0.10$ in the univariate analyses. Multicollinearity among the covariates in the final model was assessed using the variance inflation factor with a cut-off of 2 as exclusion criterion, but no covariate was excluded for this reason. In pre-planned secondary analyses, we tried to evaluate the association between TSH and singular Fried's criteria, but since for three criteria (unintentional weight loss, low physical activity level and exhaustion) we had less than 50 incident cases, we did not report these elaborations for convergence problems.

All analyses were performed using the SPSS 21.0 for Windows (SPSS Inc., Chicago, Illinois). All statistical tests were two-tailed and statistical significance was assumed for a p-value <0.05.

RESULTS

Baseline characteristics

As shown in **Figure 1**, among the 3,099 participants enrolled in the study, we initially excluded 528 participants (73 subjects were taking thyroid hormones, 187 with inadequate TSH data, 192 with TSH over or below the normality range, and 76 with no data about frailty) retaining a sample of 2,571 community-dwelling elderly subjects for the cross-sectional analyses.

Cross sectional results

The mean age of the sample was 74.1±7.0 years [range: 65-99], 60% were females. The mean TSH was 1.5±0.8 mUI/L, with significantly higher levels in women versus men (1.6±0.9 vs. 1.3±0.8 mUI/L, $p<0.0001$). At baseline, 183 (=8.3%) participants were frail.

Table 1 shows the baseline characteristics by TSH quintiles and gender. Taking men in Q3 as reference, men in Q5 were significantly older, more disabled, with lower MMSE score, but with significant higher Charlson co-morbidity score and use of medications and worse renal function (**Table 1, upper part**). Similarly, men with lower TSH levels (=Q1) were significantly less educated and with poorer monthly income and took a higher number of drugs than men in Q3. Regarding women, those in Q5 were significantly older than women in Q3, whilst participants in Q1 had a significant lower BMI, GDS and MMSE score when compared to the reference group (**Table 1, lower part**).

Figure 2 shows the prevalence of frailty according to baseline serum TSH concentrations by gender. The lowest prevalence of frailty was present in those in Q3 in both genders (=6.3%; men=4.2%, women=7.6%), while those in Q1 (=5.5% in men and =10.4% in women) and Q5 (=7.5% in men and =15.3% in women) had the highest.

Table 2 summarizes the association between baseline serum TSH and the prevalence of frailty in men and women, respectively. Taking those in Q3 as the reference group, and after adjusting our analyses for 12 potential confounders (age, BMI, smoking habits, alcohol drinking, education,

monthly income, activities of daily living, GDS, MMSE, Charlson co-morbidity score, eGFR, and number of drugs), participants in Q5 reported a significant higher odds of prevalent frailty independently from the gender (OR=1.55; 95%CI: 1.03-2.33 for men and =1.97; 95%CI: 1.59-2.45 for women) (**Table 2, upper parts**).

Table 3 reports the association between baseline serum TSH levels and the presence of individual Fried's criteria. Taking those in Q3 as reference and after adjusting for the same confounders listed for Table 2, men in Q1 had a significant higher odds of having weakness and exhaustion, whilst those in Q5 of having weakness and slow gait speed. Conversely, in women, lower TSH levels (i.e. Q1) had a significant higher odds of having all the Fried's criteria (except for exhaustion), whilst those in Q5 reported a significant higher risk of poor physical performance in terms of weakness and slow gait speed.

Follow-up data

As shown in **Figure 1**, the longitudinal analyses considered 1,732 individuals (627 were excluded due to mortality, 29 because they did not return at follow-up, and 183 were frail at baseline). During the follow-up period, 93 men and 183 women became frail (=13.6% of baseline male population and 17.4% of women).

As reported in **Figure 2,** the cumulative incidence of new cases of frailty was lowest in participants in the third quintile for women (=14.1%) and for Q1 in men (=9.0%).

Using a logistic regression analysis adjusted for potential confounders at baseline (including also the presence of pre-frailty) and taking those in the third quintile for reference, men in Q5 had an increased risk of frailty at follow-up (OR=1.37; 95%CI: 1.02-1.91) similarly to women in Q1 (OR=1.47; 95%CI: 1.21-1.78) **(Table 2, lower part**).

DISCUSSION

This is the first study showing that in a large cohort of community-dwelling older men and women, TSH is associated with prevalent and incident frailty. In men, higher TSH levels are associated with both prevalent and incident frailty, whilst in women we observed that higher TSH levels are associated with an increased presence of frailty at baseline, but lower TSH levels were associated with an increased risk of frailty in longitudinal analyses. However, when taking the individual Fried's criteria as outcome, lower TSH levels seem to be more strictly associated with these parameters than higher levels, particularly in women.

TSH is a pituitary hormone that stimulates the thyroid gland to produce hormones (FT3 and FT4). The TSH levels are controlled by FT3 and FT4 concentrations thanks to a fine mechanism of feedback. The most common endocrine alterations are due to primary thyroid dysfunctions, while a minor part is related to pituitary disorders. Given this background, we assumed that lower TSH levels may correspond to sub-clinical/clinical hyperthyroidism, whilst higher TSH levels could be representative of sub-clinical/clinical hypothyroidism.

Regarding lower TSH levels, it is known that subclinical and clinical hyperthyroidism are associated with several co-morbidities (namely osteoporosis, cardiovascular diseases and cognitive decline) also common in people with frailty.[8] However, after adjusting our analyses for these confounders, our results remained significant for incident frailty in women. Moreover, lower TSH levels were associated with a significant higher odds of having several of the single items of Fried's criteria (particularly in women) suggesting that hyperthyroidism could play a role in the development of frailty. Another hypothesis is that lower TSH levels are associated with low physical performance level[21] and since Fried's criteria are mainly based on physical performance items, it is likely that the effect of low TSH could depend on this factor. Third, low TSH, although in the range of normality, could be associated with weight loss, another characteristic considered in the Fried's definition of frailty. This finding is indirectly confirmed by our analyses, since in

women low TSH levels were associated with a 65% increased odds of having weight loss, also after considering the effect of potential confounders.

Conversely, regarding higher TSH levels, it is known that this bio-humoral condition is associated with other co-morbidities highly prevalent in frail subjects such as depression.[8] Hypothyroidism, in fact, can cause a number of symptoms (such as a feeling of tiredness, depression, fatigability) that are also typical of frailty. Moreover, hypothyroidism has been reported associated to low-grade inflammation, a condition strongly related with frailty, also if TSH is within normal ranges. [22] Finally, the subjects with high TSH levels usually have some neuromuscular and musculoskeletal symptoms[23] that are overlapping with those specific of frailty status. Again our analyses showed that in both genders, high TSH levels are associated with higher presence of weakness and slow gait speed, reinforcing the concept that this condition is important for a good physical performance level.

Indeed, TSH could be useful as biomarker of frailty, since small variations within the normal range from the reference group significantly predicted the onset of frailty. The absence of an univocal operational definition for frailty, however, makes the development of measurable biomarkers tailored to the chosen definition. Thus, verifying whether TSH maintains correlative and predictive properties using different definitions of frailty and in different populations might confirm its potential role as a biomarker.[24]

Another important finding of our work is the substantial difference between men and women in the relationship between baseline serum TSH levels and incident frailty. In men, higher TSH levels predicted incident frailty, whilst in women we observed that lower TSH levels were associated with an increased risk of frailty. Altogether these findings confirm the important gender differences as proposed by animal models[25] and confirmed in human beings[26], showing that TSH is significantly

higher in women than in men and that TSH is more responsive to thyroid function alterations in men than in women. [26]

To date, two cross-sectional studies[6,7] and one longitudinal study have not found a significant association between TSH levels and frailty.[8] Several explanations might account for this discrepancy. First, that the longitudinal study by Virgini et al.[8] followed-up 22 subjects with subclinical hyperthyroidism and 85 with subclinical hypothyroidism and thus clearly lacks power. Moreover, this study, utilized FT4 data and this may offer a potentially more complete assessment of thyroid function. Second, Virgini et al.[8] only considered men. Not only does this reduce generalizability, but frailty and thyroid dysfunctions are less common in men than women. Finally, the different definitions of frailty could play an additional role in interpreting the different results. Given the aforementioned, we consider that our representative cross sectional and cohort study is the most robust study to date to investigate the relationship between TSH and frailty, building on the previous literature.

Nonetheless, whilst our study contains novel findings, it is important to consider a number of limitations. The main limitation is that we used TSH as a marker of thyroid function. Although it is likely that to low TSH levels correspond high FT4 levels and vice versa, other more rare conditions could be misinterpreted. Moreover, in the elderly (particularly in the very old, disabled, and with several co-morbidities) it is common a condition characterized by low FT3 with increased rT3 and TSH levels[27], reinforcing the lack of thyroid hormones as main limitation of our work. In a similar way, the lack of auto-antibodies could represent another limitation for our findings. Unfortunately in the PRO.V.A. study these parameters of thyroid function were not assessed, since this study was primarily designed to explore the association between osteoarticular and cardiovascular diseases and their impact of life of older people. Second, we did not assess the changes in thyroid function over time, whilst it seems that serum TSH shifts progressively to higher levels with age, also when

considering centenarians.[28] Third, some substances that might influence thyroid function (e.g. cortisonic drugs, lithium, recent investigations with iodinated contrast material etc.) were not considered. Future research should therefore attempt to investigate the impact of these on the development of frailty by TSH levels. Fourth, the PRO.V.A. study was undertaken approximately 20 years ago. Although the methods of ascertaining both frailty and TSH remain similar, we cannot exclude a bias in our results. Finally, due to the limited number of cases, we were not able to explore the potential association between serum baseline TSH and the incidence of the individual items of Fried's criteria. Focused research regarding this topic is needed.

In conclusion, men with higher and women lower serum TSH levels are at increased risk of frailty. These findings suggest important gender differences in the association between thyroid function and frailty. Our study suggests that TSH could be used as marker for frailty since small variations significantly predict the onset of frailty. Future longitudinal studies are, however, needed to confirm our findings.

ACKNOWLEDGEMENTS

We are thankful to all interviewers, nurses, and physicians who participated in the study.

Funding source: The data collection phase of the PRO.V.A. study was supported by the Fondazione Cassa di Risparmio di Padova e Rovigo; University of Padova; the Azienda Unità Locale Socio Sanitaria 15 and 18 of the Veneto Region; and a grant from the Veneto Regional Authority (Ricerca Sanitaria Finalizzata n.156/03). The data analysis phase was also financed by a grant of the University of Padova (Population aging - economics, health, retirement and the welfare state - POPA_EHR).

Author Disclosure Statement: None of the authors have any financial arrangements, organizational affiliations or other relationships that might give rise to any conflict of interest regarding the subject matter of the manuscript submitted.

REFERENCES

1. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet*. 2013;381(9868):752-762. doi:10.1016/S0140-6736(12)62167-9.
2. Collard RM, Boter H, Schoevers RA, Oude Voshaar RC. Prevalence of frailty in community-dwelling older persons: a systematic review. *Journal of the American Geriatrics Society*. 2012;60(8):1487-1492. doi:10.1111/j.1532-5415.2012.04054.x.
3. Puts MTE. Frailty: biological risk factors, negative consequences and quality of life. January 2006. https://www.researchgate.net/publication/254824221_Frailty_biological_risk_factors_negative_consequences_and_quality_of_life. Accessed February 10, 2016.
4. Fontana L, Addante F, Copetti M, et al. Identification of a metabolic signature for multidimensional impairment and mortality risk in hospitalized older patients. *Aging Cell*. 2013;12(3):459-466. doi:10.1111/acel.12068.
5. Soysal P, Stubbs B, Lucato P, et al. Inflammation And Frailty In The Elderly: A Systematic Review And Meta-analysis. *Ageing Research Reviews*. 2016. doi:10.1016/j.arr.2016.08.006.
6. Wang GC, Talor M V, Rose NR, et al. Thyroid autoantibodies are associated with a reduced prevalence of frailty in community-dwelling older women. *The Journal of clinical endocrinology and metabolism*. 2010;95(3):1161-1168. doi:10.1210/jc.2009-1991.
7. Yeap BB, Alfonso H, Chubb SAP, et al. Higher free thyroxine levels are associated with frailty in older men: the Health In Men Study. *Clinical endocrinology*. 2012;76(5):741-748. doi:10.1111/j.1365-2265.2011.04290.x.
8. Virgini VS, Rodondi N, Cawthon PM, et al. Subclinical Thyroid Dysfunction and Frailty Among Older Men. *The Journal of clinical endocrinology and metabolism*. 2015;100(12):4524-4532. doi:10.1210/jc.2015-3191.
9. Yeap BB, Flicker L. Hormones and cardiovascular disease in older men. *Journal of the American Medical Directors Association*. 2014;15(5):326-333.

doi:10.1016/j.jamda.2013.12.004.

10. Giandalia A, Russo GT, Romeo EL, et al. Influence of high-normal serum TSH levels on major cardiovascular risk factors and Visceral Adiposity Index in euthyroid type 2 diabetic subjects. *Endocrine*. 2014;47(1):152-160. doi:10.1007/s12020-013-0137-2.

11. Corti M-C, Guralnik JM, Sartori L, et al. The effect of cardiovascular and osteoarticular diseases on disability in older Italian men and women: rationale, design, and sample characteristics of the Progetto Veneto Anziani (PRO.V.A.) study. *Journal of the American Geriatrics Society*. 2002;50(9):1535-1540. http://www.ncbi.nlm.nih.gov/pubmed/12383151. Accessed November 3, 2015.

12. Katz S, Downs TD, Cash HR, Grotz RC. Progress in development of the index of ADL. *The Gerontologist*. 1970;10(1):20-30. http://www.ncbi.nlm.nih.gov/pubmed/5420677. Accessed July 20, 2015.

13. MF F, LN R, JE H. THe mini-mental state examination. *Archives of General Psychiatry*. 1983;40(7):812. http://dx.doi.org/10.1001/archpsyc.1983.01790060110016.

14. Kørner A, Lauritzen L, Abelskov K, et al. The Geriatric Depression Scale and the Cornell Scale for Depression in Dementia. A validity study. *Nordic journal of psychiatry*. 2006;60(5):360-364. doi:10.1080/08039480600937066.

15. Katz JN, Chang LC, Sangha O, Fossel AH, Bates DW. Can comorbidity be measured by questionnaire rather than medical record review? *Medical care*. 1996;34(1):73-84. http://www.ncbi.nlm.nih.gov/pubmed/8551813. Accessed March 25, 2016.

16. Niculescu D. *Williams Textbook of Endocrinology*. Vol 4.; 2008. doi:10.4183/aeb.2008.127.

17. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *The journals of gerontology Series A, Biological sciences and medical sciences*. 2001;56(3):M146-56. http://www.ncbi.nlm.nih.gov/pubmed/11253156. Accessed August 27, 2014.

18. Sergi G, Veronese N, Fontana L, et al. Pre-frailty and risk of cardiovascular disease in

elderly men and women: the pro.v.a. study. *Journal of the American College of Cardiology*. 2015;65(10):976-983. doi:10.1016/j.jacc.2014.12.040.

19. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *Journal of psychiatric research*. 17(1):37-49. http://www.ncbi.nlm.nih.gov/pubmed/7183759. Accessed August 25, 2014.
20. GRAMBSCH PM, THERNEAU TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81(3):515-526. doi:10.1093/biomet/81.3.515.
21. Portella RB, Silva JL da C, Wagman MB, de Oliveira FP, Buescu A, Vaisman M. Exercise performance in young and middle-aged female patients with subclinical hyperthyroidism. *Thyroid : official journal of the American Thyroid Association*. 2006;16(8):731-735. doi:10.1089/thy.2006.16.731.
22. Kvetny J, Heldgaard PE, Bladbjerg EM, Gram J. Subclinical hypothyroidism is associated with a low-grade inflammation, increased triglyceride levels and predicts cardiovascular disease in males below 50 years. *Clinical Endocrinology*. 2004;61(2):232-238. doi:10.1111/j.1365-2265.2004.02088.x.
23. Monzani F, Caraccio N, Del Guerra P, Casolaro A, Ferrannini E. Neuromuscular symptoms and dysfunction in subclinical hypothyroid patients: beneficial effect of L-T4 replacement therapy. *Clinical endocrinology*. 1999;51(2):237-242. http://www.ncbi.nlm.nih.gov/pubmed/10468996. Accessed February 10, 2016.
24. Calvani R, Marini F, Cesari M, et al. Biomarkers for physical frailty and sarcopenia: State of the science and future developments. *Journal of Cachexia, Sarcopenia and Muscle*. 2015;6(4):278-286. doi:10.1002/jcsm.12051.
25. da Costa VM, Moreira DG, Rosenthal D. Thyroid function and aging: gender-related differences. *The Journal of endocrinology*. 2001;171(1):193-198.
26. Suzuki S, Nishio S, Takeda T, Komatsu M. Gender-specific regulation of response to thyroid hormone in aging. *Thyroid Research*. 2012;5(1):1. doi:10.1186/1756-6614-5-1.

27. Herrmann J, Heinen E, Kr[illegible] ll HJ, Rudorff KH, Kr[illegible] skemper HL. Thyroid function and thyroid hormone metabolism in elderly people low T3-syndrome in old age? *Klinische Wochenschrift*. 1981;59(7):315-323. doi:10.1007/BF01525000.

28. Gesing A, Lewiński A, Karbownik-Lewińska M. The thyroid gland and the process of aging; what is new? *Thyroid research*. 2012;5(1):16. doi:10.1186/1756-6614-5-16.

FIGURE LEGEND

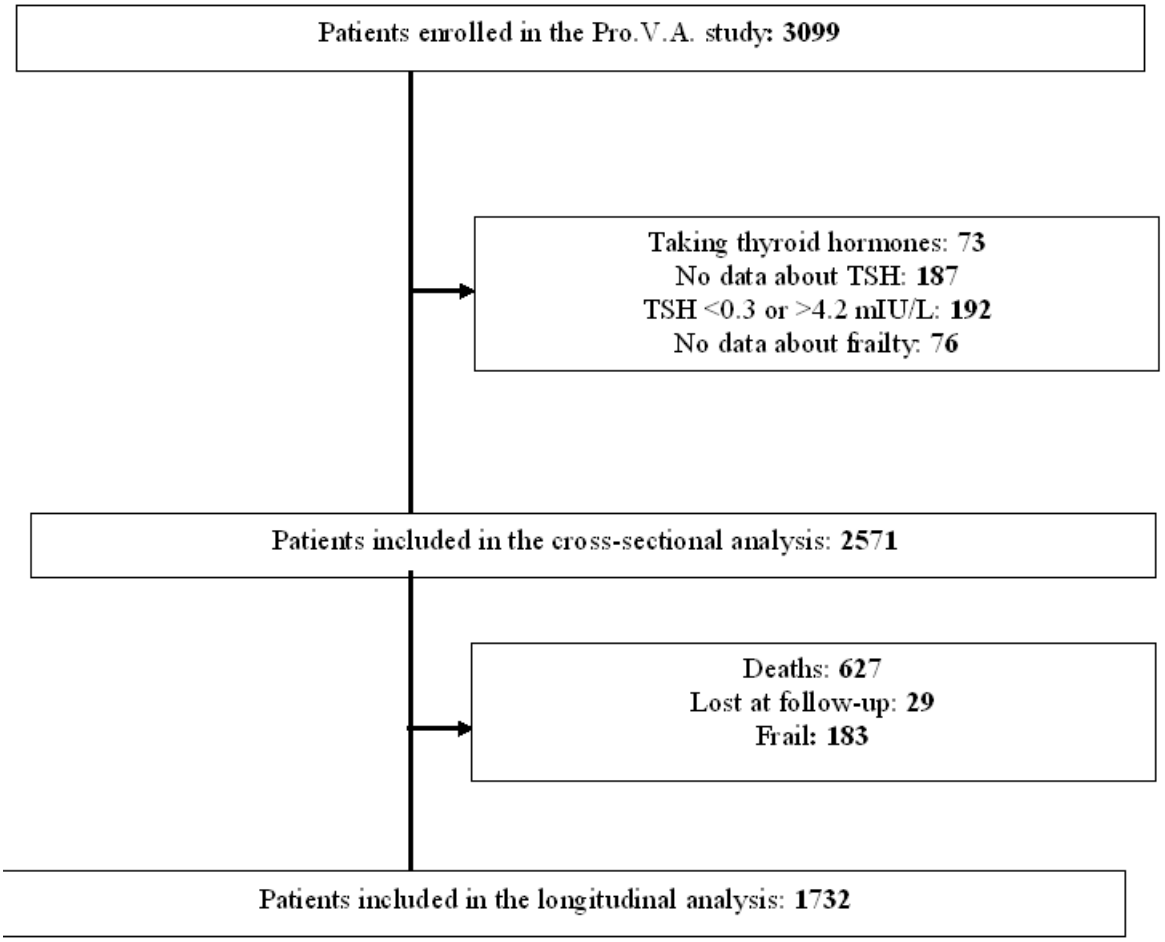

Figure 1. Flow chart of the PRO.V.A. study

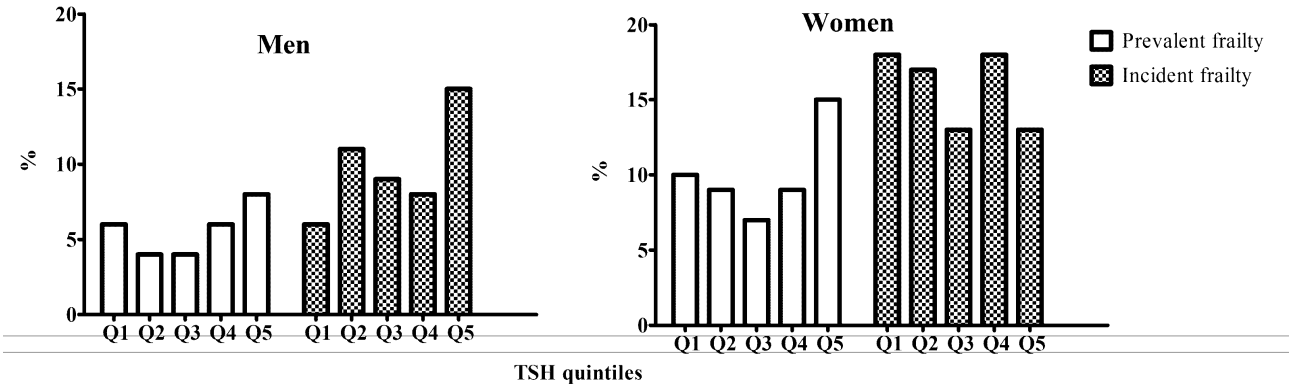

Figure 2. Prevalence and incidence of frailty by baseline thyroid-stimulating hormone (TSH) levels in men and women.

	TSH quintiles[a]				
	Men				
Participants' characteristics	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Age (years)	72.9 (6.0)	73.0 (6.4)	72.5 (6.4)	73.7 (6.6)*	74.0 (7.0)*
BMI (kg/m^2)	27.4 (4.0)	26.9 (3.7)*	27.6 (3.5)	26.8 (3.9)*	27.1 (4.0)*
Socio-economical parameters					
Current smokers (%)	15.4	14.6	16.7	25.9	20.9
Alcohol drinkers (%)	87.0	81.8	86.0	81.8	84.5
Education (>5 years) (%)	17.4*	20.7*	25.6	22.4*	23.4
Monthly income (>500 €) (%)	51.8*	56.1	55.2	54.4	56.5
ADL score	5.5 (1.2)	5.3 (1.4)*	5.5 (1.1)	5.4 (1.2)*	5.3 (1.3)*
Medical conditions					
GDS score	8.1 (4.3)	8.4 (4.8)	8.3 (4.3)	8.7 (4.8)	8.0 (4.6)
MMSE score	25.0 (5.1)*	24.4 (5.4)*	25.6 (3.9)	25.2 (4.8)	24.8 (5.0)*
eGFR (ml/min)	78.0 (18.1)	78.3 (19.5)	78.1 (20.1)	76.0 (18.6)*	72.9 (19.0)*
Charlson co-morbidity index	1.0 (1.2)	1.1 (1.2)*	0.9 (1.2)	1.1 (1.3)*	1.1 (1.3)*
Number of drugs	3.1 (1.7)*	2.9 (1.8)*	2.9 (1.6)	3.0 (1.9)	3.4 (2.1)*
	Women				
Age (years)	75.0 (7.5)	74.4 (7.1)	74.8 (7.2)	74.1 (7.1)*	75.3 (7.7)*
BMI (kg/m^2)	27.9 (4.8)*	28.2 (4.8)	28.3 (4.9)	28.5 (4.8)	28.5 (5.3)
Socio-economical parameters					
Current smokers (%)	4.0	3.2	3.6	8.5*	3.2
Alcohol drinkers (%)	56.7	58.4	58.4	57.1	58.5
Education (>5 years) (%)	11.6	11.4	10.7	15.4*	9.0
Monthly income (>500 €) (%)	26.7	34.5	33.1	37.2*	31.7
ADL score	5.0 (1.5)	5.0 (1.6)	5.0 (1.4)	5.2 (1.4)	4.9 (1.5)
Medical conditions					
GDS score	9.6 (5.7)*	10.2 (6.1)	10.2 (5.8)	9.8 (5.8)	10.0 (6.2)
MMSE score	22.7 (6.4)*	23.3 (6.2)	23.4 (5.7)	23.6 (5.8)	23.0 (6.1)
eGFR (ml/min)	67.5 (17.1)	70.7 (20.1)*	67.8 (17.0)	68.5 (22.4)*	66.9 (17.5)
Charlson co-morbidity index	1.0 (1.2)	1.1 (1.3)	1.1 (1.2)	1.0 (1.2)	1.0 (1.2)
Number of drugs	3.4 (2.0)	3.3 (1.8)	3.4 (1.9)	3.4 (1.8)	3.4 (2.0)

Table 1. Participants' characteristics by baseline thyroid-stimulating hormone (TSH) levels and gender: the PRO.V.A. study.

Notes:

[a]TSH quintiles were calculated using gender specific cut-offs: for men: 0.7, 1.0, 1.3, and 2, while for women 0.8, 1.1, 1.5, and 2.5 mUI/L.
Statistical significance: taking Q3 as reference, p-value < 0.05 was indicated with * for each quintile.
Numbers are mean values (and standard deviations) or percentages (%), as appropriate.
Age adjusted p-value were calculated with a generalized linear model for continuous and logistic regression analysis for categorical variables, respectively, using age as covariate (except for age).

Abbreviations: ADL: activities of daily living; BMI: body mass index; eGFR: estimated glomerular filtration rate; GDS: geriatric depression scale; MMSE: mini-mental state examination.

Rejuvenation Research
SERUM THYROID-STIMULATING HORMONE LEVELSAND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Table 2: Association between baseline thyroid-stimulating hormone (TSH) levels with prevalent and incident frailty by gender: the PRO.V.A. study.

	Men				Women			
	Unadjusted model	**p-value**	**Fully-adjusted model[a]**	**p-value**	**Unadjusted model**	**p-value**	**Fully-adjusted model[a]**	**p-valu**
			Prevalent frailty					
Q3	1 [reference]	-	1 [reference]	-	1 [reference]	-	1 [reference]	-
Q1	1.20 (0.85-1.70)	0.30	1.13 (0.77-1.67)	0.54	1.16 (0.94-1.42)	0.16	1.14 (0.91-1.42)	0.25
Q2	0.87 (0.60-1.25)	0.44	0.62 (0.41-1.13)	0.34	0.86 (0.69-1.08)	0.19	0.89 (0.70-1.13)	0.33
Q4	1.36 (0.94-1.95)	0.10	0.94 (0.62-1.42)	0.77	1.13 (0.91-1.39)	0.28	1.22 (0.96-1.54)	0.10
Q5	1.25 (0.86-1.79)	0.24	1.55 (1.03-2.33)	0.04	2.00 (1.65-2.44)	<0.0001	1.97 (1.59-2.45)	<0.00
			Incident frailty					
Q3	1 [reference]	-	1 [reference]	-	1 [reference]	-	1 [reference]	-
Q1	0.57 (0.41-1.06)	0.08	0.48 (0.39-1.25)	0.10	1.32 (1.12-1.56)	0.001	1.47 (1.21-1.78)	<0.00
Q2	0.95 (0.71-1.26)	0.70	0.81 (0.58-1.14)	0.23	1.14 (0.95-1.36)	0.16	1.21 (0.98-1.48)	0.07
Q4	0.99 (0.73-1.34)	0.92	0.71 (0.49-1.11)	0.07	0.87 (0.73-1.05)	0.15	0.77 (0.62-1.08)	0.11
Q5	2.02 (1.52-2.66)	<0.0001	1.37 (1.02-1.91)	0.03	0.88 (0.73-1.07)	0.20	0.94 (0.75-1.17)	0.56

Unless otherwise specified, data are presented as odds ratios and 95% confidence intervals.

[a] Fully adjusted model included: age and body mass index (both as continuous); smoking habits (current vs. former/never); alcohol drinkers (yes vs. no); education (> vs. < 5 years); monthly income (> vs. < 500 €); activities of daily living, geriatric depression, mini mental state examination scores (all as continuous); Charlson co-morbidity score; estimated glomerular filtration rate and number of drugs (both as continuous).
For incident frailty, also the presence of pre-frailty at the baseline was included as covariate.

Rejuvenation Research
SERUM THYROID-STIMULATING HORMONE LEVELS AND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
This manuscript has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Table 3. Association between baseline thyroid-stimulating hormone (TSH) levels with prevalent singular Fried's item criteria, by gender: the PRO.V.A. study.

	Q1	Q2	Q3	Q4	Q5
			Men		
Unintentional weight loss	0.61 (0.13-3.00) (0.2)	2.73 (0.76-9.79) (1.1)	1 [reference] (0.4)	**6.59 (1.87-23.12)** (1.7)	No cases (0.0)
Weakness	**1.20 (1.00-1.46)** (32.2)	1.03 (0.87-1.22) (24.8)	1 [reference] (25.5)	**1.25 (1.02-1.54)** (33.5)	**1.93 (1.58-2.36)** (39.7)
Slow gait speed	0.85 (0.67-1.08) (15.6)	**1.52 (1.21-1.92)** (24.2)	1 [reference] (14.0)	**1.42 (1.11-1.80)** (22.7)	**1.76 (1.39-2.25)** (26.2)
Low physical activity level	1.42 (0.88-2.15) (9.5)	1.03 (0.72-1.48) (6.9)	1 [reference] (6.0)	1.00 (0.82-1.28) (6.4)	1.09 (0.84-1.74) (6.8)
Exhaustion	**1.50 (1.20-1.88)** (15.4)	**1.39 (1.11-1.75)** (15.8)	1 [reference] (11.1)	1.05 (0.82-1.35) (16.7)	0.99 (0.77-1.27) (15.0)
			Women		
Unintentional weight loss	**1.65 (1.00-2.73)** (1.7)	1.04 (0.59-1.84) (2.0)	1 [reference] (1.1)	0.96 (0.54-1.84) (1.6)	1.06 (0.60-1.87) (1.4)
Weakness	**1.29 (1.14-1.47)** (51.0)	1.05 (0.93-1.19) (43.5)	1 [reference] (43.9)	0.89 (0.78-1.02) (45.0)	**1.46 (1.29-1.67)** (48.4)
Slow gait speed	**1.25 (1.09-1.43)** (42.8)	1.03 (0.89-1.18) (41.1)	1 [reference] (43.8)	**1.19 (1.03-1.37)** (42.3)	**1.39 (1.21-1.60)** (46.5)
Low physical activity level	**1.44 (1.08-1.93)** (10.9)	1.09 (0.81-1.46) (11.7)	1 [reference] (8.8)	0.82 (0.59-1.13) (7.6)	1.09 (0.81-1.48) (12.8)
Exhaustion	1.04 (0.91-1.19) (26.0)	1.00 (0.87-1.15) (26.1)	1 [reference] (22.1)	0.85 (0.73-1.25) (25.9)	1.09 (0.95-1.25) (28.2)

Unless otherwise specified, data are presented as fully-adjusted[a] odds ratios and 95% confidence intervals. The prevalence of each criterion is reported below the odds ratio.

In all the analyses participants without any Fried's criterion are taken as reference.

Rejuvenation Research
OID-STIMULATING HORMONE LEVELSAND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
viewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[a]Fully adjusted model included: age and body mass index (both as continuous); smoking habits (current vs. former/never); alcohol drinkers (yes vs. no); education (> vs. < 5 years); monthly income (> vs. < 500 €); activities of daily living, geriatric depression, mini mental state examination scores (all as continuous); Charlson co-morbidity score; estimated glomerular filtration rate and number of drugs (both as continuous).

SERUM THYROID-STIMULATING HORMONE LEVELSAND FRAILTY IN THE ELDERLY: THE PRO.V.A STUDY (doi: 10.1089/rej.2016.1872)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.